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(57) Abstract

A method of diagnosing atopy or a predisposition to atopy in an individual, which comprises demonstrating the presence of a mutation or polymorphism in a specific DNA sequence of a gene encoding the beta-subunit of the high affinity IgE receptor in the individual. Two variant DNA sequences linked with atopy are as follows: 5' GAA TTG GTA TTG ATG (SEQ ID NO: 2), 5' GAA TTG GTA GTG ATG (SEQ ID NO: 4), both commencing at nucleotide 5640 of the beta-subunit gene. The invention makes it possible for the first time to identify individuals at genetic risk of developing atopic illness.

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DIAGNOSTIC METHOD AND THERAPY

The invention relates to diagnosis of atopy or of a predisposition to atopy, and to treatment of atopic or potentially atopic individuals.

Atopy is a heterogeneous disorder characterised by prolonged and enhanced immunoglobulin E(IgE) responses to common environmental antigens, including pollens and house dust mites; it underlies the common diseases of allergic asthma and rhinitis (hay fever). The high-affinity receptor for IgE (FcεRI) binds IgE to mucosal mast cells and plays a central role in allergy (1). When allergen binds to mast cell bound IgE, FcεRI initiates a series of events leading to the cellular release of inflammatory mediators. This results in mucosal inflammation and the characteristic symptoms of wheezing, coughing, sneezing and nasal blockage.

Atopy may be detected by positive skin prick tests of common allergens, by the presence of specific serum IgE against these allergens or by elevation of the total serum IgE. These three variables are strongly correlated with each other and with the presence of symptoms. Atopy, when defined as a prick skin test response to one or more common allergens, affects up to 50% of Western populations. As a result of atopy, as many as 10% of children suffer from asthma. Atopy results from complex interactions between heterogeneous genetic and environmental factors. The factors that govern the development of generalized atopic responsiveness, a characteristic of most atopics as they respond to many allergens, probably differ from those determining allergic

response to any particular allergen or specific allergic symptoms.

Using quantitative assays for IgE response to allergens, we have observed genetic linkage between generalized atopic IgE responses and chromosome 11q in a data set which includes over 300 affected sibling-pairs (2-6). This linkage is robust to phenotype classification (6). The data suggest that 60% of families, when ascertained through a young symptomatic atopic proband, are linked to chromosome 11q (5). Notably, the sharing of alleles from chromosome 11 by atopic sibling-pairs is exclusively from maternal chromosomes (4). This observation accords with data from large epidemiological studies suggesting a maternal transmission of atopy (7-9). It is consistent with a maternal effect on fetal or neonatal immune development or with paternal genomic imprinting. The interactions of the 11q locus with other genetic loci and environmental factors in determining the atopic disease phenotype remain to be determined.Early attempts at independent replication of linkage to chromosome 11q, however, have produced variable results. Genetic heterogeneity and methodological factors, in particular the numbers of families and individuals tested, account for the discrepancies. Four studies have reported negative linkage (10-13), but two contained insufficient information to confirm or exclude linkage of atopy to the marker D11S97 on chromosome 11 (10,11). Inspection of the raw data from a third study (12) of three extended pedigrees shows a maximum lod score of 1.7 at 0 recombination in one family; the other two families show paternal inheritance and non-linkage of atopy. The fourth study, of mixed extended and nuclear families, tested linkage with the locus Int2 which is telomeric to D11S97, although atopy had previously been reported as

10% centromeric to the marker; the lod score was-2 at 10% recombination (13). In addition, none of these studies took account of the maternal linkage to chromosome 11. In contrast, data from Japan, using lod scores (14), and the Netherlands, using affected sib-pair methods (15), have confirmed linkage in families with marked symptomatic atopy. Because the atopy is a complex genetic disease, we believe that genetic linkage is more satisfactorily demonstrated and analysed using affected sibling-pair methods; these are not dependent upon an assumed mode of inheritance and control for penetrance and environmental effects (4).

In linkage mapping of atopy on chromosome 11q we have defined a confidence interval for the localisation of the atopy locus around 2 homologous genes, CD20 and the β-subunit of FcεRI (5). CD20 is a proliferation and differentiation factor in B-lymphocyte lineage whose function is not known to be related to atopic IgE responses. We have previously found that CD20 Msp1 restriction alleles (16) are not associated with atopy in children from unrelated nuclear families (odds ratio for alleles A and B = 0.95, 95%CI 0.56-1.60) (5).

The Invention

We have now established that variants of the gene encoding the beta-subunit of the high-affinity receptor for IgE are associated with atopy. Surprising results have revealed that mutations or variants in the gene alter the risk of an individual being atopic. This finding makes possible for the first time the strategy of diagnosis.

The present invention provides a method of diagnosing atopy or a predisposition to atopy in an individual, which method comprises demonstrating the presence of a mutation or polymorphism in a specific DNA sequence of a gene encoding the beta-subunit of the

high affinity IgE receptor in the individual.

In a particular embodiment, the gene is on chromosome 11q. More particularly, the specific DNA sequence is located near the commencement of exon 6 of the gene on chromosome 11q.

Gene variants have been found near the commencement of exon 6 on chromosome 11q. This exon runs from nucleotide 5640 to 5738 of the beta-subunit gene. The wild type (normal) sequence at this site, commencing with nucleotide 5640 is:

5´ GAA ATT GTA GTG ATG (SEQ ID NO: 1)

The full normal sequence of the beta-subunit gene has been published (17) and can be found in the Genbank and Embl Databases, Accesssion No. M89796.

Two variant sequences have now been identified. The first, commencing at nucleotide 5640 is:

(i) 5´ GAA TTG GTA TTG ATG (SEQ ID NO: 2)

This results in a substitution of the amino acid leucine for isoleucine at position 181 and substitution of leucine for valine at position 183.

The second variant, commencing at nucleotide 5640, is:

(ii) 5´ GAA TTG GTA GTG ATG (SEQ ID NO: 4)

This results only in substitution of leucine for isoleucine at position 181.

In the method of diagnosis according to the invention, the specific DNA sequence may thus comprise one of the above sequences (i) and (ii), or a relevant portion thereof. A relevant portion is a portion which

is different to the wild type sequence.

The method may comprise amplification of the specific DNA sequence or a relevant portion thereof.

One amplification technique which may be used is the amplification refractory mutation system (ARMS) PCR technique. Another is PCR, which may be followed by probing of the amplification products with a sequence-specific nucleic acid probe capable of annealing to a relevant portion of the amplified specific DNA sequence. Other DNA or RNA-based methods may also be used.

In the ARMS technique, at least one primer is used which anneals to a DNA sequence comprising the mutant or variant sequence, but not to the wild type sequence. Thus, only when the mutation or polymorphism is present will there be successful PCR amplification. Further confirmation may be obtained by probing or sequencing or by other known methods.

Suitable primers for amplification of sequences in exon 6 of the beta-subunit gene can be devised from the known DNA sequence, and in the case of ARMS, from the variant sequences (i) and (ii) above.

The method of diagnosis according to the invention may thus be performed on a DNA sample, but the invention is not limited to testing DNA. The method may instead be performed on a product of the specific DNA sequence, such as messenger RNA (mRNA). Or the mutation or polymorphism may be identified in cDNA made from mRNA.

Alternatively, the method may involve identifying the presence of a variant peptide or protein derived from the specific DNA sequence. For instance, antibodies raised against the variant peptide sequence may be labelled and used for <u>in vitro</u> or <u>in vivo</u> diagnosis. The variant peptide sequence can be synthesised by standard techniques eg. using an

automatic synthesiser. The antibodies can be made by administering the peptide in antigenic form to a suitable host. Polyclonal or monoclonal antibodies may be prepared by standard techniques.

The invention provides peptides corresponding to variants of exon 6 of the gene encoding the high affinity IgE receptor on chromosome 11q, and phosphorylation and glycosylation products, and characteristic fragments thereof.

Such a peptide preferably comprises the amino acid sequence:

Glu Leu Val Leu Met (SEQ ID NO: 3) or
Glu Leu Val Val Met (SEQ ID NO: 5),

or a relevant portion thereof. A relevant portion is a portion which is different to the wild type. The two above-mentioned amino acid sequences correspond to the variant nucleic acid sequences (i) and (ii).

The invention also provides antibodies to the variant peptides described above, and fragments of the antibodies. the antibodies or fragments will be useful in the method of diagnosis according to the invention, to identify protein variants.

In another aspect, the invention provides, as new chemical compounds, nucleic acids comprising the sequence (i) or (ii) above or complementary DNA or RNA.

In a particular emdodiment, the invention provides a nucleic acid comprising a first portion which corresponds substantially to the whole or part of exon 6 of the gene encoding the beta-subunit of the high-affinity receptor for IgE, which first portion includes one of the following sequences:

5' TTG GTA TTG or
5' A TTG GTA GTG (SEQ ID NO: 6) or
5' TTG GTA GTG A (SEQ ID NO: 7)

or complementary DNA or RNA, and optionally a second portion which corresponds substantially to the whole or part of an intron adjacent to said exon or complementary DNA or RNA.

Probes comprising a wild type or variant oligonucleotide or a nucleic acid as described herein, linked to a signal moiety or immobilised on a surface, are also considered to be part of the invention. Variant probes will be useful for identifying variant phenotypes and wild type probes can be used for control purposes.

Detailed Description

The invention therefore provides diagnostic tests for functional polymorphisms within and close to the beta chain gene. These tests may be used for post-natal diagnosis of an atopic predisposition, in order to carry out preventative measures against allergen sensitisation in early childhood. The tests may also identify asthmatic or other atopic subjects who respond to particular treatment modalities. The tests may also identify individuals susceptible to industrial asthma, or to the effects of cigarette smoke and other pollutants.

The recognition that the beta chain predisposes to asthma permits novel methods of treatment of asthma (and other atopic illnesses such as allergic rhinitis and eczema) directed at the beta chain, such as pharmacologic blocking of its action. The invention also provides treatments arising from recognition that variation in the beta chain is central to the atopic state, and methods for developing such treatments. Treatments may be developed for example by testing pharmacologic compounds against cell systems (eg. monkey cos cells) containing the receptor genes. Effects of pharmacologic compounds can be tested on

wild type and variant-encoded receptors, to look for compounds which eg. down-regulate the variant receptor but not the wild type receptor. High throughput screening assays will be possible. In other words, the mutant beta chain would be part of an assay to develop new drugs, or proteins to alter the receptor function . A strategy based on "antisense RNA" to block the action of the beta chain can also be envisaged.

The mutations discussed above were found in atopic individuals and their families. Initially genomic DNA was sequenced from each of the seven exons and splice sites of FcεRI-β in six atopic and six non-atopic individuals. One atopic individual was found to have a chromosome with three nucleotide substitutions in the 6th exon, resulting in Ile181Leu and Val183Leu substitutions within the 4th transmembrane domain (TM) of FcεRI-β (17) (Fig. 1). Details are given in Example 1.

The prevalence of leucine residues at positions 181 and 183 of FcεRI-β and their relationship to atopy were defined using allele specific DNA amplification (ARMS) (18), as described in Example 2. In a random patient sample, Leu181 shows association with atopy. But in accordance with the documented maternal inheritance of atopy on chromosome 11q, 11 of 24 (46%) Leu181 heterozygotes in the random patient sample were non-atopic.

Family studies were carried out to clarify the relationship between genotype and phenotype (Example 2). In each of 10 atopic families in which Leu181 was found, transmission was through the mother and a strong association between the variant and atopy was demonstrable in the children.

The strong association between maternally inherited Leu181 and atopy in a set of unrelated families indicates variants of FcεRI-β as one cause of

atopic IgE responsiveness. This is consistent with the known biological functions of the high affinity IgE receptor (1,19). FcεRI is comprised of three subunits α, β and gamma$_2$; in human, α and gamma are encoded on chromosome 1 and the β subunit on chromosome 11 (5). FcεRI is expressed on mast cells, basophils, monocytes and Langerhans cells. The receptor plays a central role in the mediation of IgE dependent allergic inflammation (1) but also in IgE metabolism and mast cell and B-lymphocyte differentiation and growth. Stimulation of FcεRI causes release from the mast cell of cytokines, including IL-4, which are implicated in the up-regulation of mast cell and helper T-cell subtype 2 (TH2) development and of IgE production by B-lymphocytes. Lung mast cells that express cell contact signals including CD40 ligand may, in the presence of IL-4, regulate local B lymphocyte IgE production independently of T lymphocytes. Variants of FcεRI-β might promote the atopic state either by enhanced release of pro-inflammatory mediators by mast cells (to cause more symptomatic disease) or by enhanced mast cell expression of IL-4 and CD40 ligand (to cause more local B lymphocyte IgE production).

In the atopic subject originally found to possess Leu181 and Leu183 variants, no other mutation was detected in full coding and splice site sequences of FcεRI-β. Alpha helical TM domains play an important part in the function of FcεRI and similar receptors in which non-ionic interactions between non-polar amino acids regulate the relationship of the helices and influence signal transduction. Mutagenicity studies on the FcεRI subunits show substituting amino acids in TM domains can cause significant changes in the receptor's expression and function (20). Single amino acid changes within TM domains of other seven-helix bundle receptors have major functional effects; these include

10-20-fold changes in ligand binding in the 5-hydroxytryptamine receptor (26). The exchanges of aliphatic amino acids (Ise-Val-Leu) within a TM of FcεRI-β parallel species-specific variants of the brain cholecystokinin-B/gastrin receptor which result in 20-fold altered affinity for benzodiazepine-based antagonists (29). It may be significant that substitution of leucines at positions 181 and 183 in human FcεRI-β generates the same sequence documented in rodents (21,22).

Our observations that 60% of families with an atopic asthmatic are maternally linked to chromosome 11 and that Leu181 occurs in 17% suggest that other variants or mutations of FcεRI-β are to be expected.

An investigation was carried out on 1004 individuals in 232 two-generation families from an Australian population (Example 3). Within this population sample, maternal inheritance of FcεRI-β Leu181/Leu183 is strongly associated with atopic IgE responses, elevated eosinophil counts, and bronchial hyper-responsiveness. Children with the variant had greater skin prick tests and RASTs to HDM than other atopic children. The variant therefore identifies a genetic risk factor for marked atopy. A 4.5% prevalencein this population implies that Leu181/Leu183 should be considered to be a polymorphism or variant of normal, rather than a mutation.

It is of note that the "Irish" variant Leu181/Leu183 was found exclusively in the Australian population, although Leu181 seems much more common in English subjects (Examples 1 and 2). This indicates possible variation between populations.

The results make it clear that, in order to interpret the presence of Leu181 or Leu181/Leu183, the maternal or paternal origin of the allele needs to be known. In the Australian study, the completely

negative skin tests and specific IgE titres of subjects who have inherited Leu181/Leu183 paternally was unexpected, given the high background level of atopy. Possible mechanisms for the maternal effect include genomic imprinting or maternal influences through the placenta or breast milk (4). A significant and opposite paternal effect, if confirmed, would favour genomic imprinting as a cause of these phenomena.

One aim of defining the genetic components of atopy has been the identification of individuals at genetic risk of developing atopic illnesses. The present results indicate that polymorphism in FcεRI-β is one factor that can be used to assign such risk. As the timing and degree of exposure to allergen in early life may determine subsequent probability of atopic disease (27), recognition of genetic susceptibility and manipulation of the environment in these individuals may result in effective prevention of illness and morbidity (28).

Reference is directed to the accompanying drawings, in which:-

Figure 1 is a schematic model of the β-subunit of FcεRI(3) demonstrating four transmembrane domains and the position of the leucine substitutions (181 and 183 as solid symbols) within the 4th transmembrane domain, and

Figure 2 shows results of ARMS testing for Leu181 in 60 nuclear families identified through an asthmatic proband. The 10 families with the variant are shown. No family was found with Leu183 variant.

EXAMPLES

Example 1

Six atopic and 6 non atopic individuals were selected for initial DNA sequence analysis.

Atopy phenotype testing.

Atopy was defined as described (30,31), by the presence of a total serum IgE elevated above normal values (Phazedym PRIST, Pharmacia), or a positive skin prick test to house dust mite or grass pollen allergens (Dome-Hollister-Stier, Spokane, USA) ≥ 2mm > a negative control, or a positive specific IgE titre > 0.35 $KU_A L^{-1}$ for the same allergens (Pharmacia CAP system). Individuals with raised total IgE alone but who were smokers were designated as unknown phenotype.

DNA sequence analysis.

DNA sequence spanning all 7 exons and their splice donor and acceptor sites of FcεRI-β was generated by PCR from genomic DNA of 6 atopic and 6 non-atopic individuals. The reaction mixture contained 1µg of genomic DNA in a buffer ($MgCl_2$ 1.5mmol L^{-1} Tris 100 mmol L^{-1}, KCl 500 mmol L^{-1}, gelatin 1mg ml^{-1}), with 200 µM of dNTPs, 0.5µl Taq polymerase, and 10% DMSO made up to a final volume of 100 µL. The primers for exons 1 to 3 (reaction 1) were: 5´-TGG GGA CAA TTC CAG AAG AAG-3´ and 5´ - CCG GAA TTC AGG TTT CTC ATG GGA TAA - 3´; and for exons 4 to 6 (reaction 2) were : 5´-TTA GGT GTC TCT CAA CCC ATC-3´ and 5´-CCG GAA TTC CTC ACA AGC CTT CTG TAC-3´; and for exon 7 (reaction 3) were: 5´-CAG CTA ACT TAG GAG GCT GAG-3´ and 5´-TAT CAG GCG AAT AAA TCT AAT GTA-3´. 25 cycles of PCR were carried out for each reaction. The products were then cut with restriction enzymes: reaction 1 used BamHI, PstI and EcoRI to give two major fragments of 0.7 and 1.7kb. The product of reaction 2 was digested with SmaI and EcoRI to yield one major fragment of 2.4 kb; reaction 3 was digested with SmaI and BamHI to give a single major fragment of 0.7 kb. The four fragments were cloned into M13 by standard methods. After checking inserts with a forward universal primer, single-strand

sequencing was carried out by the dideoxy chain termination method with the following exon-specific primers: exon 1, 5´-GTT TTC CCA GTC ACG ACG T*-3´; exon 2, 5´-GGT CAG TTA CTT GGA TGC TC-3´; exon 3, 5´-ACA GTC TAG GAC ACT AAC GC-3´; exon 4, 5´-GGA TTA CAG ACA TGA GCC AC-3´; exon 5, 5´-AGA CCG TAC GTG TTC ATG TG-3´; exon 6, 5´-GTC AGA TGG TAG GGA GAT G-3´;exon 7, 5´-GTT TTC CCA GTC ACG ACG-T*-3´ (*indicates M13 - 40 forward primer). Six clones were sequenced for each exon from each individual. Mutations were considered to be present if seen in 2 or more clones.

One atopic individual was found to have a chromosome with three nucleotide substitutions in the 6th exon, resulting in Ile181Leu and Val183Leu substitutions within the 4th TM domain of FcεRI-β, as discussed above.

Example 2

For association studies between FcεRI-β variants and atopy, two groups were studied:

(i) A random patient sample of 163 males and females aged 15 -40 years having blood counts carried out at the John Radcliffe Hospital. (ii) 60 nuclear families freshly recruited through atopic asthmatic probands under the age of 21 attending hospital or general practitioner clinics in Oxfordshire. These families had not previously been assessed for linkage to chromosome 11 markers.

Atopy phenotype testing was carried out as described in Example 1. In the random patient sample, total and allergen-specific serum IgE´s were assayed but skin prick test and clinical data were not available.

Allele specific DNA amplification (ARMS) for Leu181 and Leu183.

The Arms method applied was modified from ref.(18). For FcεRI-β, the primers to give a 237 bp band were: a universal upstream primer 5´-AAG TTA TCT ACT GCA AGT GAC GAT CTC T-3´ (SEQ ID NO: 8) together with downstream primers to detect: wild type sequence (Ile181, Val183), 5´-GGT GAG AAA CAG CAT CAT CAC TAC AAT-3´ (SEQ ID NO: 9); the Leu181 variant, 5´-GGT GAG AAA CAG CAT CAT CAA TAC CAA-3´ (SEQ ID NO: 10); the Leu183 variant, 5´-CAG AAT GGT GAG AAA CAG CAT CAT CAA-3´ (SEQ ID NO: 11). Concurrent amplification of HLA-DP sequence was used as a positive control in each reaction to give a 312 bp band. The primers were: 5´-TCA CTC ACC TCG GCG CTG CAG -3´ (SEQ ID NO: 12) and 5´-CCC TCC CCG CAG AGA ATT AC-3´ (SEQ ID NO: 13). PCR was performed in a Perkin Elmer Cetus DNA thermal cycler using a preliminary cycle (94°C denaturation for 5 min, 60°C annealing for 2 min, and 72°C extension for 2 min) and then 34 cycles (94°C for 2 min, 60°C for 2 min, and 72°C for 2 min). Amplification products underwent electrophoresis in 4% agarose gels before ethidium staining and scoring by two independent observers. Note: careful purification of genomic DNA was essential for effective ARMS testing.

Protocol.

Genotyping and phenotyping were carried out randomised and double blind. The atopy phenotype was ascribed prior to DNA analysis. Freshly extracted DNA samples from all subjects were coded in random order, obscuring all family links. The ARMS testing was performed in duplicate with positive and negative controls. The presence of Leu181 was tested and confirmed by DNA sequencing in the 10 families.

(i) In the random patient sample (Table 1),

Leu181 was found in 25 of 163 individuals (15%) of whom one was homozygous; none showed a Leu183 substitution. Associations were found between the presence of Leu181 and high total serum IgE [odds ratio (OR) 3.07 (95% Confidence Interval 1.25-7.55, Fisher's statistic (FS) = 5.96, p=0.01] and positive IgE tests to grass pollen antigen [OR 2.61 (95% CI 1.07-6.4), FS 4.48, p=0.03] but not to house dust mite antigen (OR 1.44, 95% CI 0.6-3.5). Thirteen (56%) of the Leu181 positive subjects were designated atopic (12 by positive RAST tests) and showed a mean total serum IgE of 300 kU L^{-1}; total serum IgE varies with age, race and other variables but the upper limit of normal, by association with allergen sensitization and allergic symptons, is estimated to be about 100 kU L^{-1} in non-smoking adults in Western populations.

(ii) The results from the 60 nuclear families are shown in Fig. 2. Ten (17%) of the families were found to have the Leu181 variant segregating; this was confirmed by DNA sequencing. In each family, Leu181 was maternally inherited (FS=22.2, p<0.0001). Amongst the children, Leu181 showed a strong association with atopy (all 12 children with Leu181 were atopic; whereas 10 of 12 Leu181 negative children were not non-atopic, FS=18.4, p<0.0001). Atopy was observed in a child without Leu181 in families 2 and 10 and in each instance the father also had atopy without Leu181. Eight of the 10 Leu181 heterozygous mothers (from the various parts of England and Wales) were themselves atopic. DNA was available from both maternal grandparents in two families; Leu181 was of grandmaternal origin where the Leu181 mother was atopic and of grandpaternal origin where the Leu181 mother was non-atopic. Inheritance of Leu181 from a mother is highly predictive of atopy in these ten families, all thirteen such individuals were atopic.

The phenotype in these family subjects was of marked atopy. Only 2 of 14 atopic children showed elevation of total IgE without allergen specific responses (Table 2) and many of the probands had hay fever and eczema in addition to asthma.

Example 3

A study was carried out to examine the prevalence of Leu181 and Leu181/183 in an Australian general population sample. The aim was to test if, when maternally inherited, the variants endowed a significant risk of atopy.

Subjects.

The study population consisted of 1004 subjects in 232 nuclear families from the rural coastal town of Busselton, 200 miles from the main population centre of Perth in South-Western Australia. Families were identified through adults aged 55 or under, from an electoral roll of approximately 9,000. It was emphasised that people who considered themselves normal were important to the study. However, there is known to be a high prevalence of atopy in Bussleton and other Western Australian populations.

Clinical Protocol.

Testing took place in the autumn and winter of 1992, over the three months of May, June and July. A respiratory questionnaire, based on the American Thoracic Society questionnaire but including questions on rhinitis and allergies, was administered. Skin prick testing to common allergens (Dermatophagoides pteronyssinus (HDM), rye grass, cat and dog dander, aspergillus fumigatus, alternaria alternata and negative control (Dome-Hollister-Steir, Spokane USA)) was carried out as previously described (4): wheal

diameters were calculated minus the negative control. Bronchial responsiveness to methacholine was carried out as described (23, 24): the maximum dose administered was 12 µmol. The provocative dose to produce a 20% fall in the FEVI (PD20) was estimated by linear interpolation of points on the dose-response curve. Blood was taken by venipuncture for IgE assays, eosinophil and white cell counts, and DNA studies.

Serology for IgE and white cell counts.

The total serum IgE and specific IgE to whole Dermatophagoides pteronyssinus and Phleum pratense was determined (Pharmacia CAP system FEIA, Sweden). A specific IgE RAST class 1 (≥ 0.35 KU/L) was considered positive. Eosinophil and white cell numbers were estimated by automatic counter (Western Diagnostic Laboratories, Western Australia).

DNA Testing.

DNA was obtained from peripheral blood leucocytes by phenol/chloroform extraction. FcεRI-β Leu181 detection was carried out by the Amplification Refractory Mutation System (ARMS) PCR (25) with the following oligonucleotide primers.

a) 5FU: TGT ATG TGT CAC TTT AAA AGG ACT GGT CAG (SEQ ID NO: 14).

b) 5WK: TTG TCA TTT GTT GCT GTT CAA TAG GAA GTT (SEQ ID NO: 15).

c) 3M: AAT GGT GAG AAA CAG CAT CAT CAT TAC CAA (SEQ ID NO: 16).

d) 3FU: TAA CAT ATC AGT CCT ATT ATC CCA ACC CTC (SEQ ID NO: 17).

Genomic DNA samples (0.25-0.30µg) were amplified in a total volume of 50µl containing 0.5µM of oligonucleotide primers 5FU, 3FU and 5WK, 0.1µM of 3M, 200µM dNTPs, 1 x reaction buffer (43mM KCl, 8.6mM Tris-

HCl (pH8.3), 2.5mM $MgCl_2$, 0.008% gelatin) and 2 units DNA Taq Polymerase (Boehringer Mannheim), overlaid with mineral oil. The reaction mixture (40µl) without enzyme was heated to 95°C for 5 min using a thermal cycler (Hybaid) and held at 80°C for the addition of enzyme (2 units of enzyme in 10µl of reaction buffer). Reaction conditions then followed 35 cycles of 94°C for 1 min, 60°C for 2 min, 72°C for 2 min and 1 cycle 72°C for 10 min. Amplified products were separated in a 3% (3:1 LMP agarose: Nusieve) gel containing ethidium bromide and visualised under UV light. Three bands potentially resulted from the primer combinations: 5FU-3FU gave a 459bp control band. 5WK-3FU gave a 353bp band in the presence of the "wild type" Ile 181. 3M-5FU gave a 163bp band in the presence of Leu181.

A member of each family segregating Leu181 was sequenced by the Sanger method to ensure accuracy of the PCR reaction, and to determine if Leu183 was present. The 459bp 5FU-3FU band from the above reaction was taken to second round PCR with the following internal primers 5D: (5'biotinylated) AAG GAC TGG TCA GAT GGT AG (SEQ ID NO: 18) and 3D: GGC TTC TAT CTA CCT TGT TTC (SEQ ID NO: 19). Single strand template was prepared with strepavidin-labelled magnetic beads (Dynal, Oslo, Norway) and direct solid phase sequencing followed with the sequencing primer 3GS: TCC TTT GAG TTC TTC CCC A (SEQ ID NO: 20).

Genotyping was carried out without knowledge of phenotype and vice versa.

Statistical Analysis

Differences between subjects with different FcεRI-β genotypes were estimated non-parametrically by the Mann-Whitney U test and by Kruskal-Wallis one way ANOVA (SPSS program, McGraw Hill Co., USA). Contingency table analysis, Common Odds Ratios and 95%

Confidence intervals were estimated by exact methods (STATXACT program, Cytel Corp., USA).

Results

Five hundred and two subjects were male. The parents ages were between 30 and 55 years (mean age 40.2, standard deviation (SD) 4.98) and the children between 5 and 27 (mean age 12.6, SD 4.73). Forty-five % of the parents and 43% of the children had a positive skin prick test ≥ 4mm to HDM or rye grass or both; 41% of parents and 44% of children had positive specific IgE titres (RASTs) to either HDM or grass pollen or both. Twenty-three % of the parents and 24% of the children reported wheezing or whistling from their chest in the previous year, and 8% of the parents and 14% of the children reported an attack of asthma in the same interval. Fifty % of the parents and 42% of the children reported episodic sneezing.

The assay for Leu181 failed to amplify in 5 individuals (0.5%). Of the remaining 999 subjects, 45 (4.5%) were positive for Leu181. Twenty-one of these were children; 8 (in 7 sibships) had inherited the variant paternally, and 13 (in 7 sibships) maternally. Sequencing of an individual from each family showed that in each case Leu181 was accompanied by Leu183, so that only the Leu181/Leu183 polymorphism was found in this population.

The 13 children who had inherited Leu181/Leu183 maternally were all atopic (Table 3a). Eleven had symptoms of wheeze or rhinitis or both, and a twelfth, who denied symptoms, had previous physician-diagnosed and treated asthma. Compared to the 531 other children in the population, the 13 had significantly elevated skin tests and RASTs to HDM and to grass pollen (Table 4a). The common odds ratio (OR) for a positive skin test ≥ 4mm to HDM or grass or both,

compared to other children, was 7.6 (95% confidence interval (95%CI) 1.62 - 70.8, p=0.002). The 95%CI for the OR of a positive RAST to either or both allergens was 3.1 - ∞ (p=0.001). When compared only to children with skin tests ≥ 4mm or positive RASTs or both, children with maternal Leu181/Leu183 still had greater skin tests and RASTs to HDM (p=0.005 and p=0.035 respectively).

In addition to measures of the IgE response, the eosinophil counts in the 13 children were significantly above the counts of the other children in the population, and the PD20 to methacholine was significantly lower (Table 4a). Seven children had increased bronchial responsiveness, defined as a PD20 ≤ 10μmol methacholine (23) (OR 3.75, 95%CI 1.06-14.8, p=0.014). Although the trend was for the total serum IgE to be elevated (p=0.08), the IgE levels were not significantly different from other children.

The 8 children who had inherited Leu181/Leu183 paternally were, by contrast, non-atopic, with negative skin tests and RASTs (Table 3b). Their skin tests, RASTs and eosinophil counts were significantly lower than those of other children (Table 4b).

Analysis of variance by ranks showed that maternal Leu181/Leu183, paternal Leu181/Leu183, and other children formed significantly different groups for skin tests to HDM (p=0.0000) or grass (p=0.01), or RASTs to HDM (p=0.003) or grass (p=0.01, and eosinophil counts (p=0.007).

Table 1

Associations between measures of total and specific IgE (RAST) to house dust mite (HDM) and grass pollen and the presence of Leu181 in a random sample of 163 patients

Phenotype		Leu181 -	Leu181 +	Fisher's statistic	p	Odds ratio (95% confidence interval)
Total Serum IgE	>100	30	11	5.96	0.01	3.07(1.25-7.55)
	<100	109	13			
RAST to HDM	+	46	10	0.73	ns	1.44(0.60-3.50)
	-	93	14			
RAST to Grass Pollen	+	34	11	4.48	0.03	2.61(1.07-6.40)
	-	105	13			

Table 2

The phenotype of members of ten families segregating Leu181

ID	Sex	Atopy status[a]
1.1	M	N
1.2*	F	A

Table 2 (continued)

The phenotype of members of ten families segregating Leu181

ID	Sex	Atopy status[a]
1.3*P	F	A
1.4	F	N
2.1	M	A
2.2*	F	A
2.3*P	M	A
2.4	M	N
2.5*	M	A
2.6	M	A
3.1	M	A
3.2*	F	A
3.3	M	N
3.4*P	F	A
4.1	M	N
4.2*	F	A
4.3	M	N
4.4	M	N
4.5*P	M	A
5.1	M	N
5.2*	F	N
5.3*P	F	A
5.4	F	N
6.1	M	N
6.2*	F	A
6.3*P	F	A
6.4	F	N
7.1	M	A
7.2*	F	A
7.3*P	M	A

Table 2 (continued)

The phenotype of members of ten families segregating Leu181		
ID	Sex	Atopy status[a]
7.4	F	N
8.1	M	N
8.2*	F	A
8.3	M	N
8.4*P	M	A
9.1	M	A
9.2*	F	Unknown
9.3*P	M	N
9.4*	F	A
10.1	M	A
10.2*	F	A
10.3	M	N
10.4*P	M	A
10.5	M	A

The phenotype of families are shown in Fig.2. Individuals are numbered from left to right, beginning with the parents.

*, Heterozygotes for FcεRI-β Leu181; P, Proband.
[a]A, Atopic; N, non-atopic.

Table 3a

Clinical details of children with maternally inherited *FcεRI-β Leu181/Leu183*

Pedigree	age	sex	spt[5] HDM *mm*	spt grass *mm*	RAST HDM	RAST grass	Total IgE *IU/L*	PD20[1] *μmol*	eosinophils $10^9/L$	wheeze	asthma	hay fever
6	17	f	5	5	4	3	92	NR	0.54	n	n	y
6	7	m	5	0	5	0	201	7.21	1.63	y	y	y
29	20	f	6	11	5	4	243	NR	0.58	n	n	y
29	18	f	7	0	3	0	63	8.87	0.01	n	n	y
29	14	f	7	5	4	2	166	0.19	0.44	n	y	n
61	8	f	17	0	5	0	215	1.94	1.10	y	y	y
61	14	m	8	8	5	3	550	3.18	0.70	y	y	y
95	11	1	6	4	4	1	178	6.67	0.59	n	n	y
95	10	1	3	2	4	2	137	0.14	0.66	y	y	n
162	14	2	9	4	2	2	15	NR	0.42	y	n	y
181	8	1	6	3	1	2	88	NR	0.19	y	y	n
181	7	1	4	3	2	2	235	2.5	0.23	n	n	y
209	17	2	3	0	0	1	70	NR	0.18	n	n	n

[5]spt= skin prick test
[1]NR = not reactive to maximum dose of methacholine

Table 3b

Clinical details of children with paternally inherited *FcεRI-β Leu181/Leu183*

pedi-gree	age	sex	spt[5] HDM mm	spt grass mm	RAST HDM	RAST grass	Total IgE	PD20[1] µmol	eosino-phils $10^9/L$	wheeze	asthma	hay fever
103	5	f	0	0	0	0	162	2.66	0.32	n	n	n
141	13	f	0	0	0	0	44	NR	0.05	y	n	n
150	10	f	0	0	0	0	131	1.31	0.30	y	y	y
157	6	f	0	0	0	0	117	NR	0.25	n	n	n
171	14	m	0	0	0	0	6	NR	0.10	n	n	y
171	12	f	0	0	0	0	30	NR	0.04	-	n	-
203	21	m	0	0	0	0	8	NR	0.19	n	n	n
214	19	m	0	0	0	0	80	NR	0.02	n	n	n

[5]spt= skin prick test
[1]NR = not reactive to maximum dose of methacholine

Table 4a

Mean ranks of measures of atopy in children with maternally inherited FcεRI-β Leu181/Leu183 compared to other children. A high rank indicates a high relative value for a particular parameter.

Parameter	Mann-Whitney U Test Mean Rank		z	p
	Maternal *Leu181/Leu 183* (n=13)	Others (n=531)		
spt HDM	456.19	273.21	-4.363	0.0000
spt Grass	353.23	270.52	-2.145	0.03
RAST HDM	423.15	268.81	-3.925	0.0001
RAST Grass	343.88	270.75	-1.812	ns
Total IgE	347.5	270.15	-1.756	ns
Eosinophils	356.27	261.67	-2.212	0.03
PD20	196.31	278.43	-2.183	0.03

Table 4b

Mean ranks of paternally inherited *FcεRI-β Leu181/Leu183* compared to other children.

Parameter	Mann-Whitney U Test Mean Rank		z	p
	Paternal *Leu181/Leu 183* (n=8)	Others (n=536)		
spt HDM	136.00	273.03	-2.635	0.008
spt Grass	165.00	267.54	-2.150	0.03
RAST HDM	159.00	270.66	-2.270	0.02
RAST Grass	146.00	270.86	-2.472	0.01
Total IgE	230.63	269.07	-0.697	ns
Eosinophils	141.63	261.35	-2.245	0.02
PD20	287.38	269.23	-0.390	ns

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(ii) TITLE OF INVENTION: DIAGNOSTIC METHOD AND THERAPY

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAAATTGTAG TGATG 15

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GAA TTG GTA TTG ATG 15
Glu Leu Val Leu Met
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Glu Leu Val Leu Met
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAA TTG GTA GTG ATG
Glu Leu Val Val Met
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu Leu Val Val Met
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATTGGTAGTG

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TTGGTAGTGA 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAGTTATCTA CTGCAAGTGA CGATCTCT 28

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGTGAGAAAC AGCATCATCA CTACAAT 27

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGTGAGAAAC AGCATCATCA ATACCAA 27

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CAGAATGGTG AGAAACAGCA TCATCAA 27

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TCACTCACCT CGGCGCTGCA G 21

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCCTCCCCGC AGAGAATTAC 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TGTATGTGTC ACTTTAAAAG GACTGGTCAG 30

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TAACATATCA GTCCTATTAT CCCAACCCTC 30

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AAGGACTGGT CAGATGGTAG 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGCTTCTATC TACCTTGTTT C 21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TCCTTTGAGT TCTTCCCCA 19

CLAIMS

1. A method of diagnosing atopy or a predisposition to atopy in an individual, which method comprises demonstrating the presence of a mutation or polymorphism in a specific DNA sequence of a gene encoding the beta-subunit of the high affinity IgE receptor in the individual.

2. A method as claimed in claim 1, wherein the gene is on chromosome 11q.

3. A method as claimed in claim 2, wherein the specific DNA sequence is located near the commencement of exon 6 of the gene.

4. A method as claimed in any one of the claims 1 to 3, wherein the specific DNA sequence containing the mutation or polymorphism comprises

5´ GAA TTG GTA TTG ATG (SEQ ID NO: 2) or

5´ GAA TTG GTA GTG ATG (SEQ ID NO: 4)

commencing at nucleotide 5640, or a relevant portion thereof.

5. A method as claimed in any one of claims 1 to 4, comprising amplification of the specific DNA sequence or a relevant portion thereof.

6. A method as claimed in claim 5, wherein the amplification refractory mutation system (ARMS) PCR technique is used.

7. A method as claimed in claim 5, wherein amplification is by PCR, and the amplification products are probed with a sequence-specific nucleic acid probe capable of annealing to a relevant portion of the amplified specific DNA sequence.

8. A method as claimed in any one of claims 1 to 7, performed on a sample of DNA.

9. As new chemical compounds, nucleic acids comprising the sequence

5' GAA TTG GTA TTG ATG (SEQ ID NO: 2) or

5' GAA TTG GTA GTG ATG (SEQ ID NO: 4),

or complementary DNA or RNA.

10. A nucleic acid comprising a first portion which corresponds substantially to the whole or part of exon 6 of the gene encoding the beta-subunit of the high-affinity receptor for IgE, which first portion includes one of the following sequences:

5' TTG GTA TTG or

5' A TTG GTA GTG (SEQ ID NO: 6) or

5' TTG GTA GTG A (SEQ ID NO: 7)

or complementary DNA or RNA, and optionally a second portion which corresponds substantially to the whole or part of an intron adjacent to said exon or complementary DNA or RNA.

11. A probe comprising a nucleic acid according to claim 9 or claim 10, linked to a signal moiety or immobilised on a surface.

12. A probe comprising a nucleic acid corresponding substantially to the whole or part of exon 6 of the gene encoding the beta-subunit of the high-affinity receptor for IgE, which nucleic acid includes the following sequence:

5' ATT GTA GTG,

or complementary DNA or RNA, linked to a signal moiety or immobilised on a surface.

13. The peptide corresponding to a variant of exon 6 of the gene encoding the high affinity IgE receptor on chromosome 11q, and phosphorylation and glycosylation products, and characteristic fragments thereof.

14. The peptide claimed in claim 13, comprising the amino acid sequence:

Glu Leu Val Leu Met (SEQ ID NO: 3) or

Glu Leu Val Val Met (SEQ ID NO: 5),

or a relevant portion thereof.

15. Antibodies to the peptides, phosphorylation and glycosylation products, and characteristic fragments, according to claim 13 or 14, and fragments thereof.

16. A method as claimed in claim 1, using antibodies according to claim 15 to identify a protein variant corresponding to the specific DNA sequence.

Figure 1

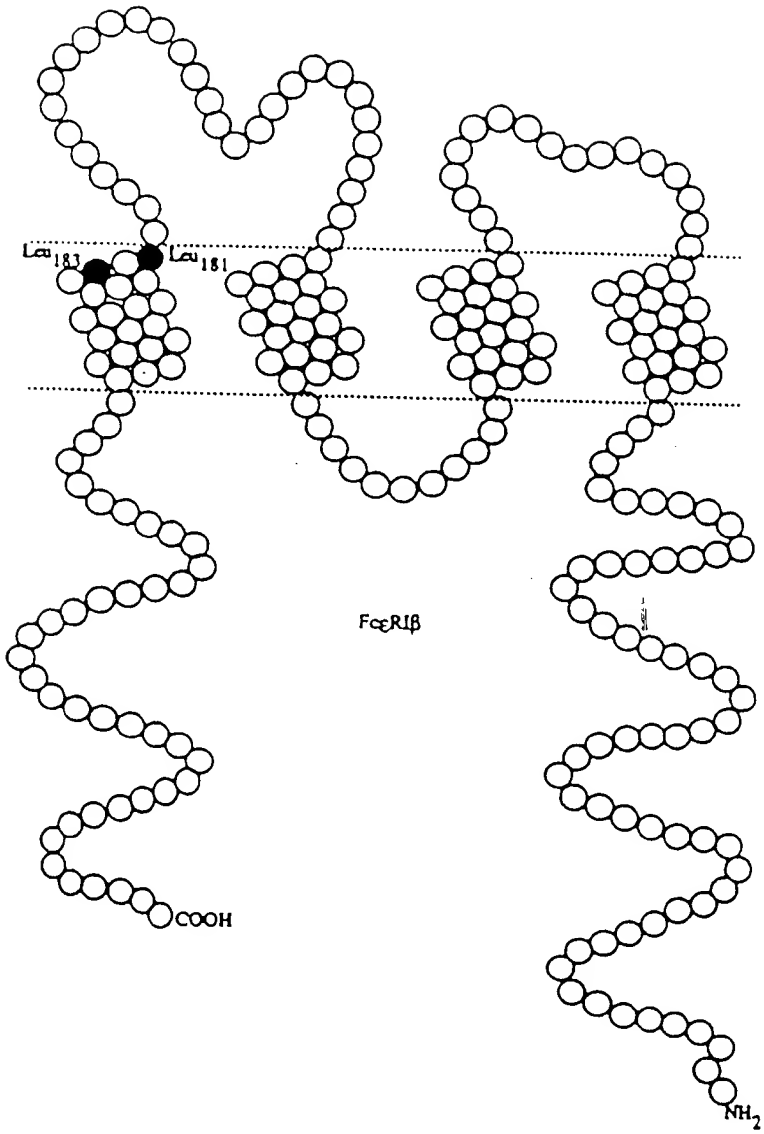
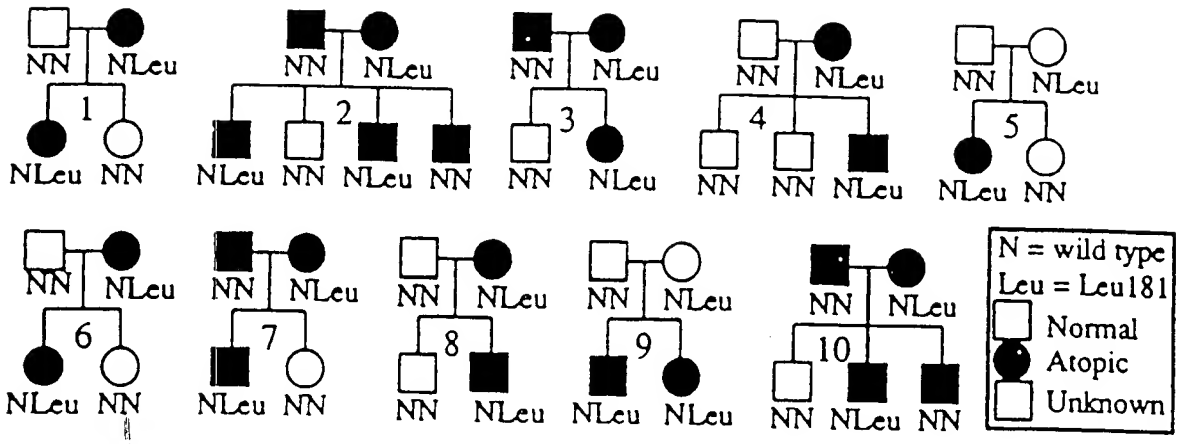


Figure 2


NN
NLeu
1
NLeu NN
2
NLeu NN NLeu NN
3
NN NLeu
4
NN NN NLeu
5
NLeu NN
6
NLeu NN
7
NLeu NN
8
NN NLeu
9
NLeu NLeu
10
NN NLeu NN
N = wild type
Leu = Leu181
Normal
Atopic
Unknown